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Comparison of the Yields of Blood Cultures Using Serum or Plasma from Patients with Early Lyme Disease

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In an initial experiment, culture-grown *Borrelia burgdorferi* was added to freshly collected uninfected human blood. This in vitro study demonstrated that more spirochetes were distributed into the plasma than into the serum fraction. In a subsequent clinical study, *B. burgdorferi* was recovered from plasma cultures of approximately 50% of 42 patients with early Lyme disease associated with erythema migrans. The rate of recovery from plasma cultures was significantly greater than that from serum cultures ($P < 0.001$).

The majority of patients with early Lyme disease present with a single, expanding, circular skin lesion called erythema migrans. Tests for detection of antibody to *Borrelia burgdorferi* are usually negative at this stage of illness (1). We have recently demonstrated that serum cultures are positive in approximately 20% of these patients (17). The volume of serum cultured correlated directly with yield. The large volume of serum cultured in that study (≥9 ml per patient) is one likely explanation for the higher culture yields compared to prior studies in which the volume of material cultured was usually <1 ml (3, 8, 10–12, 14, 15).

The purpose of the present study was to determine if EDTA-preserved plasma is preferable to serum as a source of culture material for *B. burgdorferi*.

Experiment 1. Experiment 1 was designed to determine if there is a reduction in the number of *B. burgdorferi* organisms detected in serum compared to EDTA-plasma when a large inoculum of *B. burgdorferi* is directly introduced into blood samples. *B. burgdorferi* strain 297 was grown in 50 ml of BSK medium. The BSK medium was modified by us (13) from the formula devised by Barbour (3). This BSK medium formulation did not contain antibiotics or gelatin and was supplemented with 6% normal rabbit serum (Sigma Chemical Co., St. Louis, Mo.). When late log phase was reached, the culture was centrifuged at 2,200 × *g* for 30 min to pellet the spirochetes. The pellet was resuspended in 2 ml of BSK medium, and the number of spirochetes present in a 10-μl aliquot was determined using acridine orange and a microscope equipped for fluorescence microscopy (see below). The concentration of spirochetes was adjusted to an inoculum of $\geq 10^8$/ml. This high inoculum concentration was used in order to permit visual detection of spirochetes when they were introduced into fresh whole blood (see below).

Whole blood from healthy volunteers seronegative for *B. burgdorferi* antibodies was obtained by phlebotomy and placed into both 9.5-ml EDTA-blood collection tubes and 9.5-ml blood collection tubes lacking any anticoagulant. (Three volunteers donated blood during the course of the experiment.) Immediately thereafter, 0.5 ml of the *B. burgdorferi* preparation was injected into each blood tube and the tubes were inverted four or five times to mix the spirochetes and blood thoroughly. The blood tubes were then placed upright in a test tube rack at room temperature and allowed to stand for 1.5 to 2 h to simulate clinic procedures. The blood tubes were then centrifuged at 260 × *g* for 15 min. Aliquots (10 μl) were mixed with 10 μl of acridine orange, and the spirochetes were counted by fluorescence microscopy. The mean number of spirochetes present in five fields at a magnification of ×400 was determined, and the number of spirochetes per milliliter was calculated based on the formula devised by Fieldsteel et al. for performing microscopic counts of spirochetes in liquid medium (7).

Experiment 2. In EDTA-blood collection tubes, three 3-ml samples of plasma were collected from untreated adult patients with a clinical diagnosis of erythema migrans that had been established at the Westchester Medical Center (Valhalla, N.Y.) in 1999. All patients satisfied the surveillance criteria for Lyme disease of the Centers for Disease Control and Prevention (5). In addition, three 3-ml samples of serum were col-

TABLE 1. Comparison of concentrations of *B. burgdorferi* in plasma and serum

Replicate no.[a]	Concn[b] in:		Plasma/serum concn ratio
	Plasma	Serum	
1	4.000	0.072	55.56
2	1.400	0.040	35.00
3	1.300	0.056	23.21
4	1.400	0.032	43.75
5	1.500	0.012	125.00
6	1.500	0.012	125.00
7	0.960	0.008	120.00
8	0.580	0.012	48.33
9	3.300	0.160	20.63
10	4.100	0.100	41.00
11	0.150	0.024	6.25
12	0.100	0.052	1.92

[a] Each replicate represents paired serum and plasma samples from healthy volunteers seronegative for antibody to *B. burgdorferi*. Freshly collected blood, both with and without the anticoagulant EDTA, was spiked with the same number of *B. burgdorferi* microorganisms.

[b] Concentrations are expressed as numbers of *B. burgdorferi* organisms (10^8) per milliliter.

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TABLE 2. Comparison of plasma (EDTA) and serum cultures for recovery of *B. burgdorferi* in 42 adult patients with erythema migrans[a]

Parameter	Plasma	Serum	*P* value
No. of culture-positive patients/total (%)	20/42 (48)	7/42 (17)	0.005
No. of culture positive samples/total (%)	42/126 (33)	16/126 (13)	<0.001
No. of culture-positive patients with all 3 samples positive/total (%)	9/20 (45)	4/7 (57)	0.68
No. of culture-positive patients with 2 of 3 samples positive/total (%)	4/20 (20)	1/7 (14)	1.0
No. of culture-positive patients with 1 of 3 samples positive/total (%)	7/20 (35)	2/7 (29)	1.0

[a] Three 3-ml aliquots of plasma (9 ml) and three 3-ml aliquots of serum (9 ml) were cultured per patient.

lected in sterile blood collection tubes without anticoagulant. In total, 9 ml of plasma and 9 ml of serum were collected by a single venipuncture from each patient. Both plasma and serum were separated by centrifugation at 260 × *g* for 15 min. Within 3 h of the time of collection, each 3-ml aliquot of either plasma or serum was inoculated into a 70-ml screw-cap plastic flask containing 60 ml of BSK medium prepared as described above.

Cultures were incubated at 32 to 33°C for up to 12 weeks. The cultures were examined by fluorescence microscopy at 2 weeks and thereafter at 2- to 4-week intervals. Sampling for each culture was done as follows. A 10-μl aliquot of culture material was mixed with 10 μl of an acridine orange staining solution (100 μg/ml in phosphate-buffered saline [pH 7.41]). Ten microliters of this mixture was placed on a slide overlaid with a coverslip and examined microscopically (magnification, ×400). A minimum of 20 high-power fields were viewed for the presence of spirochetes. Confirmation that the visualized spirochetes were *B. burgdorferi* was done by performing PCR on a sample of the culture medium as previously reported (16).

To remove nonborrelial microorganisms selectively, contaminated cultures were filtered twice, first through a 0.45-μm-pore-size filter (Nalgene; Nalge Co., Rochester, N.Y.) and then through a 0.2-μm-pore-size filter (Nalgene) (9).

Statistics. Fisher's exact test, two tailed, was used for comparisons of proportions. The Wilcoxon signed-ranks test was used for paired data that were not normally distributed.

In experiment 1, in which blood samples were spiked with high concentrations of *B. burgdorferi*, the number of microorganisms observed in plasma was significantly higher than that observed in serum (Table 1) ($P = 0.002$). The number of *B. burgdorferi* bacteria in plasma was 1.9 to 125 times larger than the number in serum. The median plasma-to-serum ratio of the number of *B. burgdorferi* bacteria per milliliter was 42.

In experiment 2, 20 (48%) of 42 patients had a positive serum or plasma blood culture, including 8 (67%) of the 12 patients with multiple erythema migrans lesions and 12 (40%) of the remaining 30 patients with a solitary skin lesion ($P = 0.17$). Serum was culture positive for 7 (17%) of the 42 patients, whereas EDTA-plasma cultures yielded *B. burgdorferi* for 20 (48%) patients ($P = 0.005$) (Table 2). Forty-two (33%) of the 126 3-ml plasma samples were culture positive for *B. burgdorferi*, compared to 16 (13%) of 126 3-ml serum samples ($P < 0.001$). All culture-positive patients had a positive plasma culture, and plasma was the only culture-positive sample for 13 (31%) of the 42 patients. None of the plasma or serum cultures was contaminated.

Of the 20 patients with positive plasma cultures, 7 (35%) were positive on only a single 3-ml plasma sample. This finding suggests that culturing of more than 9 ml of plasma per patient may be associated with even higher rates of recovery of *B. burgdorferi*.

These experiments have shown that 3-ml aliquots of EDTA-plasma from untreated adult patients with erythema migrans are significantly more likely to yield *B. burgdorferi* on culture than 3-ml samples of serum ($P < 0.001$). The most likely explanation for the lower rate of recovery from serum is that more spirochetes are trapped in a blood clot than in centrifuged erythrocytes. Consistent with this hypothesis, a greater-than-1-log reduction in the number of *B. burgdorferi* bacteria was demonstrated in serum compared to plasma in an in vitro study using spiked samples of fresh whole blood. Whether spirochete trapping during clot formation is merely a mechanical event or is related to binding of *B. burgdorferi* to activated platelets (6) or to another specific clot component is unknown. Alternatively, substances released during the clotting process might adversely affect the viability of *B. burgdorferi*.

The higher yield of plasma cultures compared with serum cultures is consistent with prior observations on detection of DNA of *B. burgdorferi* in blood components by PCR. Goodman et al. (8) studied 76 patients with erythema migrans and were able to amplify *B. burgdorferi* DNA in 0.5 ml of EDTA-plasma of 14 (18.4%) of the patients. PCR testing of serum, however, was negative in at least 9 of these 14 patients.

In summary, our results demonstrate that the yield of blood plasma cultures in early Lyme disease is approximately 50%, a rate comparable to or higher than those of many other common infectious diseases, such as pneumococcal pneumonia (2). Plasma is preferable to serum as a source of culture material.

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REFERENCES

1. **Aguero-Rosenfield, M., J. Nowakowski, D. F. McKenna, C. A. Carbonaro, and G. P. Wormser.** 1993. Serodiagnosis in early Lyme disease. J. Clin. Microbiol. **31:**3090–3095.
2. **Austrian, R., and J. Gold.** 1964. Pneumococcal bacteremia with special reference to bacteremic pneumococcal pneumonia. Ann. Intern. Med. **60:** 759–776.
3. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57:**521–525.
4. **Benach, J. L., E. M. Bosler, J. P. Hanrahan, et al.** 1983. Spirochetes isolated from the blood of two patients with Lyme disease. N. Engl. J. Med. **308:** 740–742.
5. **Centers for Disease Control and Prevention.** 1997. Case definitions for infectious conditions under public health surveillance: Lyme disease (revised 9/96). Morbid. Mortal. Weekly Rep. **46**(Suppl. RR-10)**:**20–21.
6. **Coburn, J., J. M. Leong, and J. K. Erban.** 1993. Integrin AII_bB_3 mediates binding of the Lyme disease agent *Borrelia burgdorferi* to human platelets. Proc. Natl. Acad. Sci. USA **90:**7059–7063.
7. **Fieldsteel, A. H., F. A. Becker, and J. G. Stout.** 1977. Prolonged survival of virulent *Treponema pallidum* (Nichols strain) in cell-free and tissue culture systems. Infect. Immun. **18:**173–182.
8. **Goodman, J. L., L. F. Bradley, A. E. Ross, et al.** 1995. Bloodstream invasion in early Lyme disease: results from a prospective, controlled, blinded study using polymerase chain reaction. Am. J. Med. **99:**6–12.
9. **Jobe, D. A., S. M. Callister, and R. F. Schell.** 1993. Recovery of *Borrelia burgdorferi* by filtration. J. Clin. Microbiol. **31:**1896–1898.
10. **Nadelman, R. B., C. S. Pavia, L. A. Magnarelli, and G. P. Wormser.** 1990. Isolation of *Borrelia burgdorferi* from the blood of seven patients with Lyme disease. Am. J. Med. **88:**21–26.
11. **Nadelman, R. B., I. Schwartz, and G. P. Wormser.** 1994. Detecting *Borrelia burgdorferi* in blood from patients with Lyme disease. J. Infect. Dis. **169:**410–411.

12. **Rawlings, J. A., P. V. Fournier, and G. J. Teltow.** 1987. Isolation of *Borrelia* spirochetes from patients in Texas. J. Clin. Microbiol. **25:**1148–1150.
13. **Schwartz, I., G. P. Wormser, J. J. Schwartz, D. Cooper, P. Weissensee, A. Gazumyan, E. Zimmermann, N. S. Goldberg, S. Bittker, G. L. Campbell, and C. S. Pavia.** 1992. Diagnosis of early Lyme disease by polymerase chain reaction amplification and culture of skin biopsies from erythema migrans lesions. J. Clin. Microbiol. **30:**3082–3088.
14. **Steere, A. C., R. L. Grodzicki, and A. N. Kornblatt.** 1983. The spirochetal etiology of Lyme disease. N. Engl. J. Med. **308:**733–740.
15. **Wallach, F. R., A. L. Fornil, J. Hariprashad, et al.** 1993. Circulating *Borrelia burgdorferi* in patients with acute Lyme disease: results of blood cultures and serum DNA analysis. J. Infect. Dis. **168:**1541–1543.
16. **Wormser, G. P., G. Forseter, D. Cooper, et al.** 1992. Use of a novel technique of cutaneous lavage for diagnosis of Lyme disease associated with erythema migrans. JAMA **268:**1311–1313.
17. **Wormser, G. P., J. Nowakowski, R. B. Nadelman, S. Bittker, D. Cooper, and C. Pavia.** 1998. Improving the yield of blood cultures for patients with early Lyme disease. J. Clin. Microbiol. **36:**296–298.